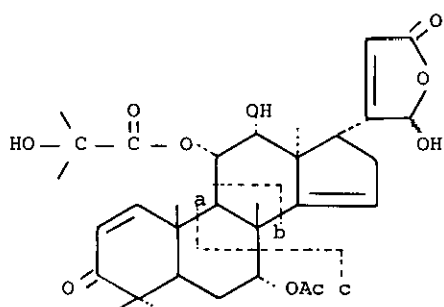


ISOLATION OF A NEW TETRANORTRITERPENOID FROM *AZADIRACHTA INDICA* A.JUSS (MELIACEAE)

Salimuzzaman Siddiqui*, Shaheen Faizi, Tariq Mahmood, and
Bina Shaheen Siddiqui
H.E.J. Research Institute of Chemistry
University of Karachi, Karachi-32, Pakistan

Abstract — A new γ -hydroxybutenolide tetranortriterpenoid named as isonimbocinolide has been isolated from the acidic fraction of the fresh, undried leaves of *Azadirachta indica* A.Juss (neem) and its structure established through chemical and spectral studies. This fraction showed insect growth regulating properties against mosquitoes (*Aedes aegypti*).

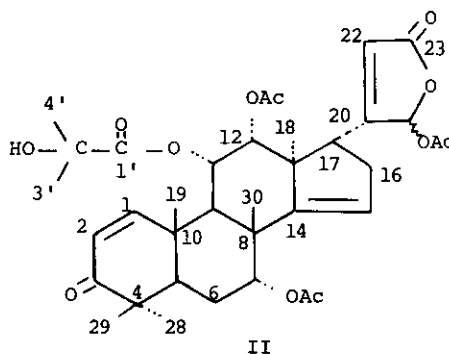
In pursuance of studies in the constituents of fruits^{1,2} and leaves³ of *Azadirachta indica* two tetranortriterpenoids γ -hydroxybutenolides have been isolated from the acidic fraction of fresh, undried neem leaves. The structure of one of these has been determined as I through chemical and spectral studies, while the other butenolide has been identified as isonimocinolide, the structure elucidation of which along with that of its isomer nimocinolide has recently been communicated.⁴ Isonimbocinolide (I)



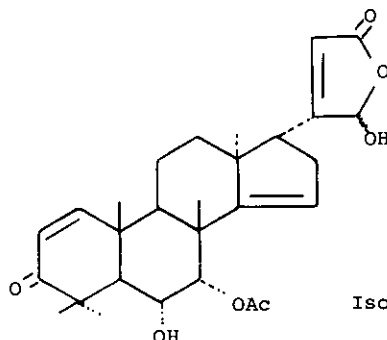
Isonimbocinolide (I)

Fragment d = b-AcOH

Fragment e = c-AcOH



II



Isonimocinolide

is of potential biological importance since preliminary experiments carried out on the acidic fraction have shown that it is capable of disturbing the metamorphic growth of mosquitoes (*Aedes aegypti*) and produces larval-pupal intermediates. Moreover, the other γ -hydroxybutenolides have also been shown to possess the insect growth regulating⁴ and insect antifeedant properties.⁵

Isonimbocinolide (I) was obtained from the acidic fraction of the ethanolic extract of neem leaves, following a tedious course of isolation procedure recorded in the experimental. It has molecular formula $C_{32}H_{42}O_{10}$ (high resolution mass) and showed maxima at 233 nm in the uv spectrum. Its ir spectrum showed peaks at $3500-3200\text{ cm}^{-1}$ (-OH), 1760 cm^{-1} (α, δ -unsaturated γ -lactone), 1740 cm^{-1} (ester carbonyl), 1665 cm^{-1} (cyclohexenone), 1640 and 820 cm^{-1} (trisubstituted double bonds). A pair of doublets at δ 7.08 and 5.90 ($J=10.1\text{ Hz}$) attributable to H-1 and H-2 respectively, and a one-proton signal at δ 5.55 for H-15 in ^1H -nmr spectrum of I, indicated that rings A and D of I are identical with those of isonimbocinolide.⁴ The presence of 21-hydroxy-20-(22)-butene- γ -lactone side chain was shown by the signals at δ 6.01 (H-21), 5.95 (H-22) in the ^1H -nmr spectrum, chemical shifts at δ 158.0 (C-20), 98.2 (C-21), 120.0 (C-22) and 170.2 (C-23) in ^{13}C -nmr spectrum (vide Table) and a fragment at m/z 486.2661 ($C_{28}H_{38}O_7$) in the mass spectrum, resulting from the loss of the C-17 side chain. Moreover; the ^1H -nmr spectrum showed signals at δ 4.05 (d, $J=2.3\text{ Hz}$), 5.45 (dd, $J=12.3$ and 2.3 Hz) and 5.42 (m, $W_{1/2} = 5.8\text{ Hz}$) suggesting the presence of a hydroxy and two ester functions, which have to be located in rings B and C. One of the ester functions which was shown to be acetoxy by a methyl signal at δ 2.16 in the ^1H -nmr spectrum was placed at C-7 on biogenetic grounds^{6,7}, which was supported by significant fragments a-e in the mass spectrum of I. The observations recorded so far indicated the composition of the second ester function as $C_4H_7O_3$, which was also born out by a fragment at m/z 483.2352 ($C_{28}H_{35}O_7$) resulting from the loss of $C_4H_7O_3$. The structure of this ester function was deduced as $(CH_3)_2C(OH)-\overset{O}{\underset{\text{O}}{\text{C}}}-O-$ from the presence of the quaternary carbinyl carbon (δ 78.9) in the ^{13}C -nmr, absence of any other carbinyl proton in the ^1H -nmr spectrum and the formation of only the triacetate II. The location of the secondary hydroxyl and the ester function could be established through the homonuclear decoupling experiments. Thus irradiation of the signal at δ 4.05 collapsed the double doublet at δ 5.45 to a doublet

($J=12.3\text{Hz}$), while irradiation at δ 5.45 resulted in the collapse of the doublets at δ 4.05 ($J=2.3\text{Hz}$) and δ 2.73 ($J=12.3\text{Hz}$) to each singlet. On the other hand, on irradiation at δ 2.73, the double doublet at δ 5.45 was collapsed to a doublet ($J=2.3\text{Hz}$). These findings led to the partial structure $\text{CH}-\overset{|}{\text{CH}}(\text{OCOR})-\text{CH}(\text{OH})-$, conclusively showing that the ester function is located at C-11 and the hydroxyl function at C-12. The W_x of H-7 and the coupling constants of H-11 and H-12 showed that the substituents at C-7, C-11 and C-12 have α disposition. In the light of the above discussion, structure I has been assigned to isonimbocinolide, which was substantiated by various fragments observed in the mass spectrum and the chemical shifts in the ^{13}C -nmr spectrum. The double signals observed for C-15 to C-17 and C-20 to C-23 (vide Table) indicated that it is also epimeric at C-21 as observed in isonimocinolide (loc.cit).

Stereochemistry of various centers of isonimbocinolide (I) has been established through NOESY experiment, which showed the connectivities of H-18 with H-22, H-3' and H-4'; H-9 with H-3' and H-4'; H-30 with H-7, H-11, H-12 and H-19; H-1 with H-2; and H-7 with H-29. These observations exhibited typical trans A/B ring junction with H-18 on the α side of the molecule. The spatial proximity of H-22 with H-18 showed that the side chain at C-17 has α disposition.

The isolation of isonimbocinolide (I) as a uniform constituent deserves special comments. Preparative tlc (silica gel; benzene-acetone 8:2) of the acidic fraction (0.38% on the dry weight basis) gave a uniform band marked as A-3, which was subjected to structural investigations when its ^1H and ^{13}C -nmr spectral data recorded on 300MHz instrument showed that it is a mixture. After a great deal of effort, it could be separated into two components marked as A-3' and A-3" on plates coated with aluminium oxide (chloroform-methanol 95:5). The nmr spectral data of A-3' indicated that it was still not uniform, and it could eventually be separated into two compounds X and Y through preparative tlc (aluminium oxide; petroleum ether-ethyl acetate 1:1) and their structures elucidated as isonimocinolide (loc.cit) and isonimbocinolide (I, yield 0.4% on the wt. of total acid fraction) respectively. It is noteworthy that preliminary biological testing carried out on the acidic fraction showed that it is capable of disturbing the metamorphic growth of mosquitoes (*Aedes aegypti*) and produces larval-pupal intermediates. Detailed investigations on this fraction as well as on the uniform constituent, isonimbocinolide, are in progress.

Table ^{13}C -NMR Spectral Data of Isonimbocinolide

Carbon No.	δ	Carbon No.	δ
1	157.0	16	33.3 33.4
2	126.3	17	52.8 52.9
3	206.0	20	158.0 158.5
4	45.0 ^a	21	98.2 99.5
5	35.6	22	120.0 ^b 120.1
6	26.6	23	170.2 170.4
7	73.8	1'	174.0
8	44.8 ^a	2'	78.9
9	46.5	3'	31.3
10	34.9	4'	31.7
11	76.5		169.0
12	68.2		20.6
13	50.1	CMe	20.6
14	156.7		21.4
15	119.6 ^b		21.7
	119.7		25.5
			31.9

a,b: values may be interchanged.

EXPERIMENTAL

Melting points were recorded in glass capillary tubes and are uncorrected. Ir and uv spectra were measured on JASCO IRA-I and Pye-Unicam SP-800 spectrometers respectively. Mass spectra were recorded on Finnigan MAT 112 and 312 double focusing mass spectrometers; exact masses have been measured through peak matching. ^1H and ^{13}C -nmr (broad band and gated spin echo) spectra, and NOESY experiments (pulse delay 2 sec., mixing time 0.5 sec.) were run on Bruker Aspect 3000 NMR spectrometer. ^{13}C -nmr spectral assignments have been made partly through spin echo spectrum and partly through a comparison of chemical shifts with the published data for

similar compounds.^{4,5,8,9} Assignments of various protons particularly in the high field region, have been made through homonuclear proton decoupling and two dimensional experiments (COSY, NOESY and J-resolved). The purity of samples was checked on tlc (silica gel and aluminium oxide).

Isolation of isonimbocinolide (I)

Fresh, undried, uncrushed neem leaves (40 Kg) collected from Karachi region, were repeatedly percolated with ethyl alcohol at room temperature. Removal of the solvent from the combined extracts under reduced pressure gave a dark green thickish residue, which was partitioned between ethyl acetate and water. The ethyl acetate layer was repeatedly extracted out with 4% Na₂CO₃ to separate the acidic fraction. The Na₂CO₃ layer was acidified and shaken out with ethyl acetate, dried, charcoaled and successively eluted with ethyl acetate and methanol-benzene (1:1). The residue obtained on removal of the solvent from the ethyl acetate eluate, was divided into petroleum ether soluble and insoluble fractions. The latter on preparative tlc (silica gel; benzene-acetone 8:2) yielded three components A-1, A-2 and A-3, the latter two being the major constituents. A-3 was further separated into A-3' and A-3'' through preparative tlc (aluminium oxide; chloroform-methanol 95:5) and the former ultimately resolved into two pure crystalline compounds X and Y through chromatography on plates coated with aluminium oxide (petroleum ether-ethyl acetate 1:1). Recrystallization of isonimbocinolide Y (I) from chloroform formed rods mp 80-81°C, $[\alpha]_D^{22} = 25^\circ$ (CHCl₃). High resolution MS m/z (%): 586.2780 (M⁺, calcd. for C₃₂H₄₂O₁₀: 586.2776) (2), 486.2661 (C₂₈H₃₈O₇) (20), 483.2352 (C₂₈H₃₅O₇) (3), 468.2467 (C₂₈H₃₆O₆) (6), 466.2357 (C₂₈H₃₄O₆) (5), 424.2281 (C₂₆H₃₂O₅) (22), 406.2165 (C₂₆H₃₀O₄) (12), 364.1483 (C₁₉H₂₄O₇, fragment a) (6), 262.1529 (C₁₆H₂₂O₃, fragment b) (5), 222.1340 (C₁₃H₁₈O₃, fragment c) (37), 202.1330 (C₁₄H₁₈O, fragment d) (10), 162.1025 (C₁₁H₁₄O, fragment e) (10) and 137.0969 (C₉H₁₃O, ring A) (8). ¹H-nmr (300 MHz, CDCl₃) δ: 7.08 (1H, d, J=10.1Hz, H-1), 6.01 (1H, m, H-21), 5.95 (1H, d, J_{22,17}=1.4Hz, H-22), 5.90 (1H, d, J=10.1Hz, H-2), 5.55 (1H, dd, J_{15,16β}=2.9Hz, J_{15,16α}=1.4Hz, H-15), 5.45 (1H, dd, J_{11β,9}=12.3Hz, J_{11β,12β}=2.3Hz, H-11β), 5.42 (1H, m, W_{1/2} = 5.8Hz, H-7), 4.05 (1H, d, J_{12β,11β}=2.3Hz, H-12β), 3.70 (1H, m, ex. with D₂O, OH), 2.91 (1H, ddd, J_{17,16β}=8.0Hz, J_{17,16α}=9.1Hz, J_{17,22}=1.4Hz, H-17), 2.73 (1H, d, J_{9,11β}=12.3Hz, H-9), 2.65 (1H, ddd, J_{gem}=15.0Hz, J_{16α,15}=1.4Hz, J_{16α,17}=9.1Hz, H-16α), 2.49 (1H, ddd, J_{gem}=15.0Hz, J_{16β,15}=2.9Hz, J_{16β,17}=8.0Hz, H-16β), 2.16 (3H, s, OAc), 1.93 (1H, dd, J_{5,6α}=2.5Hz, J_{5,6β}=11.5Hz, H-5), 1.70 (2H, m, H-6), 1.62 (2H, m, ex. with D₂O, 2 x OH), 1.31 (6H, s, H-3' and H-4'),

1.28 (3H, s, H-28), 1.27 (3H, s, H-30), 1.18 (3H, s, H-29), 1.16 (3H, s, H-19) and 0.94 (3H, s, H-18).

The compound X was identified as isonimocinolide through comparison of its spectral data with those of an authentic sample (loc.cit).

Acetylation of I to II

To a solution of I (6mg) in pyridine (0.5ml), acetic anhydride (1ml) was added and the reaction mixture kept overnight at room temperature. On usual work up, II was obtained as a crystalline solid which on recrystallization from chloroform formed needles, mp 65-67°C; uv λ_{max} (MeOH) nm: 210, 225; ir ν_{max} (CHCl₃) cm⁻¹: 3350, 1760, 1740 br., 1675, 1640 and 820. HRMS m/z (%): 670.3009 (M⁺, calcd. for C₃₆H₄₆O₁₂: 670.2987) (4), 550.2555 (C₃₂H₃₈O₈) (2), 508.2459 (C₃₀H₃₆O₇) (8), 468.2477 (C₂₈H₃₆O₆) (5), 447.2112 (C₂₈H₃₁O₅) (8) and 408.2272 (C₂₆H₃₂O₄) (6). ¹H-nmr (300 MHz, CDCl₃) δ : 7.09 (1H, d, J=10.0Hz, H-1), 6.87 (1H, m, H-21), 6.00 (1H, m, H-22), 5.93 (1H, d, J=10.0Hz, H-2), 5.45-5.38 (4H, m, H-7, H-11, H-12 and H-15), 2.76 (1H, m, H-17), 2.66 (1H, d, J=12.6Hz, H-9), 2.50 (2H, m, H-16), 2.17, 2.03, 2.00 (each 3H, s, 3 x OAc), 1.94-1.65 (2H, m, H-5 and H-6), 1.60 (1H, m, OH), 1.32 (6H), 1.27 (6H), 1.18, 1.17 and 0.94 (s, 7 x CH₃).

ACKNOWLEDGEMENT

For the biological results our thanks are due to Dr.S.N.H.Naqvi and coworkers from the Toxicology Laboratory of Zoology Department, University of Karachi. One of us (Tariq Mahmood) wishes to express his grateful thanks to Hamdard Foundation Pakistan for providing a research fellowship during the course of this work.

REFERENCES

1. S.Siddiqui, S.Faizi and B.S.Siddiqui, Heterocycles, 1984, **22**, 295.
2. S.Siddiqui, B.S.Siddiqui and S.Faizi, Planta Medica, 1985, No.6, 473.
3. S.Siddiqui, B.S.Siddiqui, S.Faizi and T.Mahmood, Phytochemistry, 1984, **23**, 2899.
4. S.Siddiqui, S.Faizi, T.Mahmood and B.S.Siddiqui, J.Chem.Soc., Perkin Trans.1, (in press).
5. W.Kraus and W.Grimminger, Nouv.J.Chim., 1980, **4**, 651.
6. D.Lavie and E.C.Levy, Tetrahedron, 1971, **27**, 3941.
7. G.P.Cotterrell, T.G.Halsall and M.J.Wrigglesworth, J.Chem.Soc., Chem.Commun., 1967, 1121.
8. W.Kraus and R.Cramer, Tetrahedron Lett., 1978, 2395.
9. Von M.Guex and C.Tamm, Helv.Chim.Acta, 1984, **67**, 885.

Received, 9th December, 1985